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BRIEF REPORT

Inflammation and Immune Activation in Antiretroviral-Treated Human Immunodeficiency Virus Type 1–Infected African Infants and Rotavirus Vaccine Responses

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Biomarkers of inflammation and immune activation were correlated with rotavirus vaccine responses in 68 human immunodeficiency virus type 1 (HIV-1)–infected (and 116 HIV-exposed but uninfected (HEU) African infants receiving pentavalent rotavirus vaccine (RV5) in a clinical trial. Prevaccination, HIV-1+ infants had significantly higher concentrations of interferon γ (IFNγ), interleukin1β, interleukin 2, interleukin 6, interleukin 10 (IL-10), and soluble CD14 compared with HEU infants. Postvaccination concentrations of neutralizing antibodies to RV5 were negatively correlated with prevaccination concentrations of IL-10 (RV5 surface proteins G1 and P1) and IFNγ (G1) in the HIV-1+ infants, whereas antirotavirus immunoglobulin A (IgA) levels were not. Heightened inflammation and immune activation in HIV-1+ infants did not alter IgA responses associated with protection from rotavirus disease.

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In human immunodeficiency virus type 1 (HIV-1)–infected (HIV-1+) adults, heightened inflammation and immune activation as a consequence of microbial translocation from impaired integrity of the gut mucosa are associated with increased non-AIDS morbidity and mortality [1] and lower responses to vaccines [2]. HIV-1+ children are known to have poorer responses to routine childhood vaccines [3], including live oral polio virus vaccine [4]. African infants have lower response rates and antibody titers to the pentavalent rotavirus vaccine (RV5) when compared with European or Latin American children [5], possibly due to a heightened inflammatory state from coinfections. The effect of inflammation and immune activation on immune responses to vaccines among HIV-1+ infants has not been investigated.

In a randomized, double-blind, placebo-controlled clinical trial (International Maternal Pediatric Adolescent AIDS Clinical Trials [IMPAACT] P1072) of the safety and immunogenicity of RV5 in HIV-1+ and HIV-1–exposed but uninfected (HEU) African infants, plasma concentrations of inflammatory cytokines and soluble CD14 (sCD14) were quantified and correlated with the humoral response to RV5 measured in the parent trial [6].

METHODS

Study Design

The study was conducted in 4 countries (Botswana, Tanzania, Zambia, and Zimbabwe) before rotavirus vaccination was part of the national immunization program. This analysis included infants who received all 3 study doses, had plasma samples available for analysis of the inflammatory and immune activation markers, and included 184 (91%) of 202 enrolled infants (68 HIV-1+ and 116 HEU). The first dose of vaccine was administered between 4 to <15 weeks of age, followed by second and third doses ≥28 days after the prior dose, with the final dose administered by ≤32 weeks of age.

Quantitation of Inflammation and Immune Activation

Cytokines commonly used to assess the inflammatory state in adult HIV-1 infection [7] (interferon γ [IFNγ], interleukin 1β [IL-1β], interleukin 2 [IL-2], interleukin 4 [IL-4], interleukin 6 [IL-6], interleukin 8 [IL-8], interleukin 10 [IL-10], interleukin 12p70 [IL-12p70], interleukin 13 [IL-13], and tumor necrosis factor α [TNFα]) were quantified at study entry (prevaccination) and 21 days after the first vaccine dose using the ultrasensitive 10-plex human cytokine kit (V-Plex Proinflammatory Kit) from Meso-Scale Discoveries (Rockville, MD). Testing at additional time points was not performed due to lack of sample availability for the entire cohort. The limit of detection for each cytokine was determined from analysis of the standard curves included in each run. Samples were measured in duplicate, and the average concentrations were determined. To minimize run-to-run variation, the same reagent lot was used to test all samples. To minimize bias, all samples were run in a blinded fashion.

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The soluble marker of monocyte/macrophage activation sCD14, was measured in plasma at study entry, 21 days after the first vaccine dose, and 14 and 42 days after the third vaccine dose, with an enzyme linked immunosorbent assay (sCD14 Quantikine Enzyme-Linked Immunoassay Kit, R&D Systems, Minneapolis, MN). Optical densities of analytes were measured using a microtiter plate reader (VersaMax Plus ROM, version 1.21, SoftMax, Sunnyvale, CA) and values quantified in GraphPad Prism version 5 (GraphPad Software, La Jolla, CA) to yield concentrations in picograms per milliliter based on the standard curve. Samples were measured in duplicate, and average concentrations were determined.

Vaccine Responses to Pentavalent Rotavirus Vaccine

Levels of serum neutralizing antibodies to the surface proteins (G1, G2, G3, G4, and P1 [A]) of the vaccine virus and total serum antirotavirus immunoglobulin A (IgA) to RV5 were determined in the parent trial [8] at baseline and 14 days after the third study dose. Stool antirotavirus IgA antibody (copro-antibody) levels were also measured at baseline and 21 days after each of the 3 study doses [8].

Statistical Methods

Baseline characteristics were compared by HIV-1 infection status using Wilcoxon rank sum tests for continuous data and chi-square tests for categorical data. Some markers had substantial proportions of measurements below the lower limit of detection, which varied between assay runs. These left-censored values were set to the lower detection limits for descriptive summaries. Because marker distributions were skewed, analyses were performed on a $\log_{10}$ scale and transformed back for presentation of results. Marker distributions were compared by HIV-1 infection status and other categorical covariates using Wilcoxon rank sum tests. Associations with continuous covariates were assessed using Spearman correlations. To account for the left censoring, multivariable models were fit using censored normal regression. A significance level of 5% was used to establish statistical significance. We did not adjust for multiple comparisons. Results should be viewed as exploratory and hypothesis generating in nature.

RESULTS

Study Population

Table 1 summarizes demographic, virologic, and immunologic characteristics and the antiretroviral prophylactic and treatment histories. Median ages at study entry for the HIV-1+ and HEU infants were 93 and 82 days, respectively. Similar proportions were breastfed and received the oral polio vaccine on the same day as the first dose of RV5. Median (Q1, Q3) percentages of CD4+ T cells at screening were lower in the HIV-1+ infants (31% [24%, 37%]) compared with the HEU infants (37% [32%, 45%]; $P < .001$). HIV-1+ infants had significantly lower World Health Organization (WHO) weight-for-age Z scores compared with the HEU infants (median of −1.5 vs −0.7; $P = .001$). Among the HIV-1+ infants, 63 of 68 (93%) had recently initiated combination antiretroviral treatment (ART) with a median (Q1, Q3) duration of 6 (0, 12) days prior to receiving vaccine. The 5 infants not on ART at study entry initiated ART within 28 days of the first vaccine dose. In the 65 HIV-1+ infants with plasma viral load (pVL) measured at study entry, the median pVL was 4.6 $\log_{10}$ copies/mL, and 61 (94%) had detectable HIV-1 RNA

Table 1. Participant Characteristics

Characteristic	HIV-1 infected (n = 68)	HIV-1 exposed but uninfected (n = 116)	*P* value
Female, no. (%)	38 (56%)	63 (54%)	.84[a]
Age at randomization, d	93 (85, 96)	82 (74, 93)	<.001[b]
Received ARV for PMTCT, no. (%)	47 (69%)	105 (91%)	<.001[a]
Ever breastfed prior to entry, no. (%)	43 (63%)	74 (64%)	.94[a]
Receipt of OPV with first vaccine dose, no. (%)	52 (76%)	89 (77%)	.97[a]
CD4% at screening	31 (24, 37)	37 (32, 45)	<.001[b]
Receipt of ART at randomization, no. (%)	63 (93%)	NA	NA
Lopinavir/ritonavir-based cART, no. (%)	46 (68%)	NA	NA
Nevirapine-based cART, no. (%)	17 (25%)	NA	NA
None, no. (%)[c]	5 (7%)	NA	NA
Duration of ART at randomization, days	6 (0, 12)	NA	NA
pVL>400 copies/mL at entry, no. (%)[d]	61 (94%)	NA	NA
HIV-1 RNA, $\log_{10}$copies/mL, median (Q1, Q3)	4.6 (3.5, 5.7)	NA	NA
WHO weight-for-age Z score, median (Q1, Q3)	−1.5 (−2.4, −0.2)	−0.7 (−1.3, −0.1)	.001[b]
WHO height-for-age Z score, median (Q1, Q3)	−1.1 (−2.1, −0.2)	−0.9 (−1.8, 0.0)	.23[b]

[a]Chi-square test.

[b]Wilcoxon tests.

[c]All human immunodeficiency virus type 1–infected infants started antiretroviral therapy within 28 days of entry.

[d]Only 65 of 68 human immunodeficiency virus type 1 (HIV-1)–infected infants had HIV-1 viral load results at entry.

Abbreviations: ART, antiretroviral therapy; ARV, antiretroviral; cART, combination antiretroviral therapy; HIV-1, human immunodeficiency virus type 1; NA, not applicable; OPV, oral poliovirus vaccine; PMTCT, prevention of mother to child transmission; pVL, plasma viral load; WHO, World Health Organization.

at >400 copies/mL. Following the third dose of RV5, 64 of the 68 HIV-1+ infants (median age, 179 days; median duration of ART, 105 days) had pVL measured; 32 of 64 (50%) had reached pVL of ≤400 RNA copies/mL.

Inflammation and Immune Activation Profiles Before and After Pentavalent Rotavirus Vaccine

HIV-1+ infants had significantly higher prevaccination concentrations of 7 (IFNγ, IL1-β, IL-2, IL-6, IL-8, IL-10, and TNFα) of the 10 cytokines measured compared with the HEU infants (Figure 1). In bivariate (adjusted for HIV-1 status) censored normal regression models, age at study entry (above or below the median age of 90 days) and ever having exposure to breastmilk were significantly associated with elevated concentrations of at least 1 inflammatory cytokine at study entry. In a multivariable model that included HIV-1 status and these 2 covariates, concentrations of IFNγ, IL1-β, IL-2, IL-6, and IL-10 remained significantly higher in the HIV-1+ infants compared with the HEU infants (ranging from 1.65-fold for IL-1β to 2.20-fold for IL-6; Supplementary Table 1). In HIV-1+ infants, higher concentrations of IFNγ, IL-2, IL-10, and sCD14 were associated with higher pVL, with an 8%–23% increase for each $\log_{10}$ increase in pVL. Also in the HIV-1+ infants, lower WHO weight-for-age *Z* scores were associated with higher concentrations of IFNγ, IL-1β, IL-2, IL-8, and IL-10 (Supplementary Figure 1). There were no significant increases relative to baseline in any of the cytokines 21 days after the first vaccine dose, in either the HIV-1+ or HEU infants, or in those receiving vaccine versus placebo (data not shown).

Plasma concentrations of sCD14 were also significantly higher in the HIV-1+ infants compared with the HEU infants at study entry (Figure 1). This difference remained significant after adjusting for age and exposure to breastmilk (Supplementary Table 1). In a multivariable model in the HIV-1+ infants adjusted for breastfeeding, WHO weight-for-age *Z* scores, and HIV-1 RNA, higher pVL was associated with higher sCD14 concentrations (Supplementary Figure 1). By the last study visit,

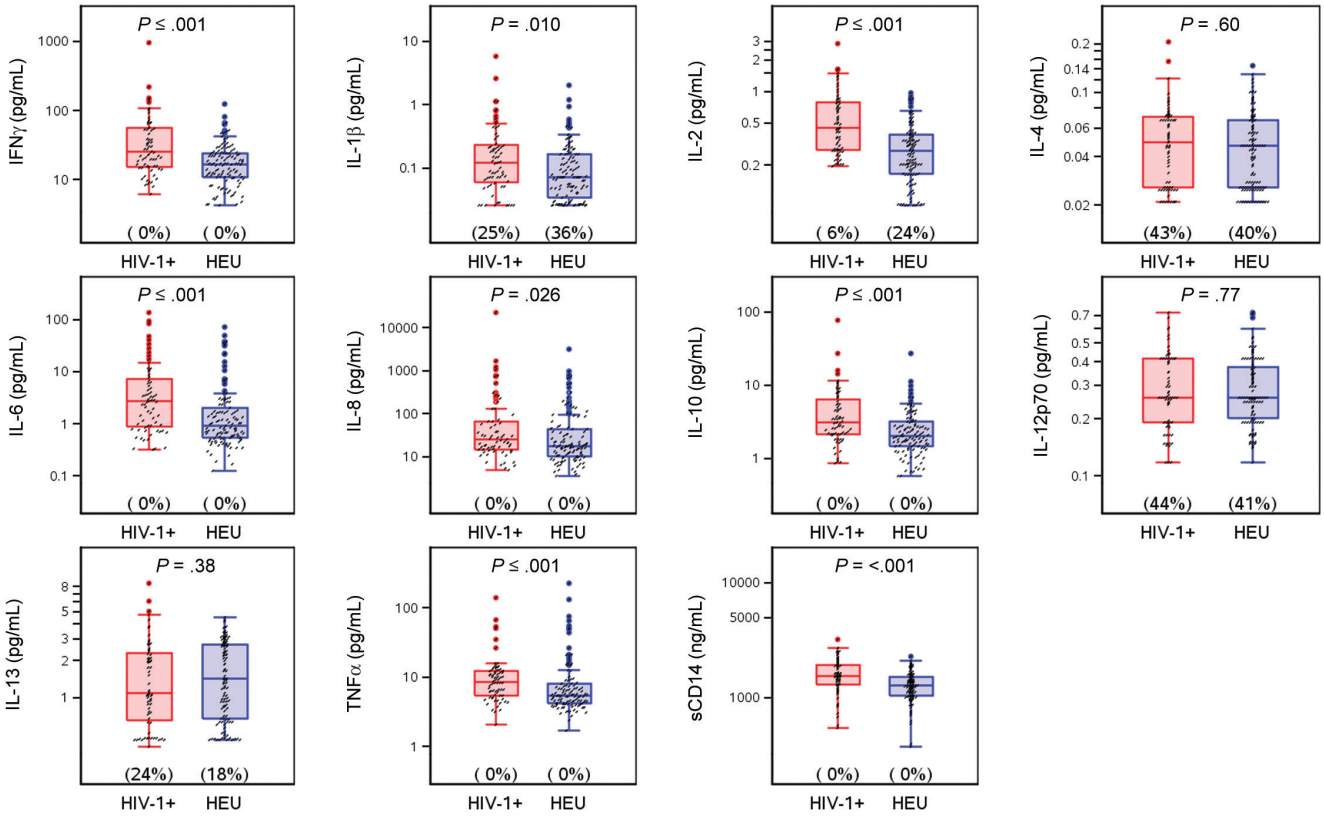

Figure 1. Baseline concentrations of plasma cytokines (interferon γ [IFNγ], interleukin 1β [IL-1β], interleukin 2 [IL-2], interleukin 4 [IL-4], interleukin 6 [IL-6], interleukin 8 [IL-8], interleukin 10 [IL-10], interleukin 12p70 [IL-12p70], interleukin 13 [IL-13], and tumor necrosis factor α [TNFα]) and soluble CD 14 (sCD14) in human immunodeficiency virus type 1–infected (HIV-1+) and HIV exposed but uninfected (HEU) African infants prior to receipt of pentavalent rotavirus vaccine (RV5) or placebo. Boxplots (showing individual data points, median [Q1, Q3], lower and upper fences [1.5 interquartile range]) of baseline concentrations of plasma cytokines. The median (minimum, maximum) limit of detection for each cytokine was as follows: IFNγ (0.45 [0.33, 1.16]), IL-1β (0.03 [0.03, 0.21]), IL-2 (0.10 [0.08, 0.23]), IL-4 (0.03 [0.02, 0.07]), IL-6 (0.09 [0.07, 0.14]), IL-8 (0.09 [0.05, 0.20]), IL-10 (0.10 [0.04, 0.13]), IL-12p70 (0.15 [0.09, 0.42]), IL-13 (0.47 [0.40, 0.98]), TNFα (0.08 [0.05, 0.12]) pg/mL. The lower limit of detection of the assay for sCD14 was 125 pg/mL. Differences between HIV-1+ (n = 68) and HEU (n = 113) infants were assessed using Wilcoxon rank sum tests. The percentage of measurements with values below the lower limit of detection is shown in parentheses.

sCD14 concentrations did not differ significantly by receipt of vaccine or placebo in either the HIV-1+ or HEU infants.

Correlates of Entry Plasma Biomarkers and Vaccine-Induced Humoral Responses

In the HIV-1+ infants, prevaccination plasma concentrations of IFNγ were negatively correlated with postvaccination neutralizing titers to the surface protein G1 ($r = -0.44$; $P = .009$) (Supplementary Figure 2). Prevaccination IL-10 concentrations were also negatively correlated with postvaccination neutralizing titers to the surface proteins G1 ($r = -0.39$; $P = .02$) and P1 ($r = -0.37$; $P = .03$). However, none of the markers of inflammation or immune activation at study entry in the HIV-1+ infants were significantly correlated with serum or stool antirotavirus IgA concentrations after vaccination. Among the 35 HIV-1+ infants who received RV5 and had HIV-1 RNA results available after the third vaccine dose, there were no statistically significant differences in serum IgA or neutralizing titers to the surface proteins in the 18 who achieved HIV-1 RNA ≤400 copies/mL on ART compared with the 17 who were not yet suppressed and had pVLs >400 copies/mL (data not shown).

DISCUSSION

Our study highlights the early presence of inflammation and immune activation in perinatal HIV-1 infection, with high circulating concentrations of Th1 (IFNγ), proinflammatory (IL-1β, IL-6), and pleiotropic cytokines (IL-2, IL-10), as well as a heightened state of monocyte activation reflected by sCD14 concentrations, when compared with HEU infants. The heightened inflammation and immune activation was associated with pVL concentrations and is consistent with previous reports of associations between sCD14 concentrations and pVL in HIV-1+ infants [9], implicating plasma viremia as a factor in the early pathogenesis of inflammation and immune activation in HIV-1 infection.

Importantly, despite significant differences in inflammation and immune activation at the start of the immunization series in HIV-1+ and HEU African infants, no significant differences in levels achieved in serum neutralizing or IgA antibodies to RV5 were observed [8]. However, negative correlations between pre-vaccination cytokine concentrations and neutralizing antibodies to the surface proteins G1 and P1 (IL-10) and G1 (IFNγ), in the HIV-1+ infants were observed. While, serum IgA responses to rotavirus vaccines have been correlated with protection from natural infection and lowered disease severity, no threshold levels of protection for the serum neutralizing antibody titers to the surface proteins (G1, G2, G3, G4, and P1) in rotavirus vaccines are yet established [10, 11]. However, because the majority of neutralizing antibodies typically belong to the IgG class, we cannot rule out an effect of HIV-1–associated inflammation and immune activation on humoral responses to other routine childhood vaccines, for which there are known target concentrations associated with immune protection. This observation may become clinically relevant in the future because the magnitude and perhaps the affinity, avidity, and/or the persistence of vaccine-induced antibodies generated during periods of inflammation may be altered; it needs further investigation. These findings differ from a small study in HIV-1+ adults, where an association between inflammation and immune activation and poorer responses to influenza vaccine was reported [2]. HIV-1+ adults typically start ART after much longer durations of infection compared with the HIV-1+ infants in this study, during which time their immune systems are likely to undergo more damage that may ultimately impact vaccine responses. Whether this observation will change with more widespread implementation of early treatment of adults will require further study [12].

In our study, administration of the oral, live-attenuated vaccine RV5 in combination with oral polio vaccine to ART-treated infants did not lead, in the short term, to noticeable increases in inflammation and immune activation in the HIV-1+ or HEU vaccinees compared with placebo recipients. Our study was, however, limited by lack of HIV-1–unexposed, uninfected infants as controls. Immunologic abnormalities and increased rates of severe infections in HEU compared with HIV-unexposed infants have been described [13–15]. Furthermore, longitudinal assessment of the concentrations of plasma cytokines throughout the vaccination period to further assess changes in cytokines on ART and with sequential exposure to oral viral vaccine antigens was not performed due to sample availability. Moreover, HIV-1+ infants had not reached steady-state levels of virologic control because they had recently initiated ART, making the interpretation of the kinetics of inflammation and immune activation levels during the study challenging. Nevertheless, this is the first study to demonstrate, in perinatally infected infants, no overt effect of HIV-1–associated inflammation and immune activation on the generation of serum and copro-IgA antibody responses to rotavirus vaccine known to confer protection from rotavirus disease. The findings are promising for immunization with oral vaccines for this vulnerable population, although the long-term effects on quality and durability of the vaccine responses need further evaluation.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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